PROPOSAL FOR CONTRACT
BETWEEN
THE COUNCIL FOR TOBACCO RESEARCH - USA
AND
MICROBIOLOGICAL ASSOCIATES
FOR
STUDIES WITH A PULMONARY EMPHYSEMA
ANIMAL MODEL SYSTEM.

Date: September 16, 1974

RESEARCH CONTRACT PROPOSAL

TITLE: "Studies with a Pulmonary Emphysema Animal Model System."

TO: Council for Tobacco Research
110 East 59th Street
New York, New York 10022

FROM: Microbiological Associates, A Division of Dynasciences Corporation
4733 Bethesda Avenue
Bethesda, Maryland 20014

DATE: September 16, 1974

Vincent L. Duwet

Vincent Duwet
Vice President, Contracts and Administration

TABLE OF CONTENTS

OBJECTIVES

The ultimate objective would be to have a small mammal with a genetic susceptibility to the dilation of air spaces distal to the bronchiole, clinically defined as emphysema. Such a model system is needed to define the effects of an overlay of air pollutants, dusts and smoking. These effects can be used to show whether dust, air pollution or smoke constitutents overlayed upon the natural genetic susceptibility, would lead to the centrilobular localized form of emphysema commonly occurring in man.

This proposed contract would initiate a breeding colony composed of subfamilies of BALB/c mice. Inbreeding of selected parents would be directed toward high or low incidence of emphysema. Further, a holding colony would be maintained and research directed toward defining the parameters of lung anatomical and physiological changes with age, tryptic enzyme inhibition serum levels and specific serum lung anti-collagen, and anti-elastin directed antibody responses.

INTRODUCTION

Thurlbeck[1], in a review paper, analyzes the difficulties in the differential diagnosis of chronic obstructive lung disease. Approximately 20% of adult human males have chronic bronchitis; apparently two-thirds of this group also have emphysema. It is uncommon to find adult lungs entirely free of emphysema at necropsy and approximately 50% of adults have "significant" defined centrilobular and/or panacinar emphysema at necropsy.

Research into the pathogenesis of emphysema, a chronic pulmonary disease restricted to dilation of air spaces distant to the terminal bronchiole, has been limited by lack of a suitable natural small mammal model of the disease.

A number of artificially induced models of emphysema have been reported. Recently Snider et al[2] reported a centrilobular emphysema in rat lungs, associated with fibrosis, experimentally induced by cadmium chloride aerosol. Krehl[3] induced panlobular emphysema through increasing resistance to bronchial air flow with a ball valve. Tura[4] subjected rats to a forced swim daily for 90 days, Gross[5] introduced papain intratracheally and Giles[6] introduced papain into rat lungs in an aerosol. It has been reported that emphysema is a major cause of disability in racing dogs[7]. Equine pulmonary emphysema, as a result of broncho-pulmonary mold allergy, has been reported[8]. Carlson[9] showed that high doses of 3-methylindole, a natural trytophane metabolite, given

intragastrically, result in emphysematous lesions in goats and cattle. Nettesheim[10] reported panlobular emphysema in hamsters as a result of chromate dust inhalation. Subacute NO_2 induced lesions of rat lungs were noted by Freeman[11], and SO_2 inhalation results in a generalized panlobular emphysema[12]. The relationship between experimentally induced emphysema in cattle, rats or dogs and naturally occurring pulmonary disease in humans is not clear. To date no animal model analogous to the congenital $alpha_1$ trypsin deficiency[13] associated with some human panlobular emphysema has been shown, although Chan[14] has defined $alpha_1$ antitrypsin inhibitors in different strains of mice.

Dr. Louise Rabstein, a veterinary pathologist at Microbiological Associates, undertook a review of slides from over 900 mice of nine different strains between 3 and 33 months of age from the long term holding colonies at Walkersville, Maryland. The most consistent finding of emphysema was in a pedigree BALB/c colony which had been maintained since 1968. All breeding within the colony was brother to sister matings. Rather than maintaining the pedigree as a single line of descendants, a number of sub-strains were developed. In 1971, this was reduced to seven separate sub-strain families (Families G, L, S, X, Y, 1 and 2). The studies initiated by Dr. Rabstein have been continued by Dr. Bernard Sass, Veterinary Pathologist.

Mice in the Pedigreed BALB/c colony are allowed to live their maximum life span. They are examined weekly for evidence of palpable enlargements. All moribund animals are sacrificed and a complete necropsy performed. Dead animals, if not decomposed, are also necropsied. Following processing of tissues by the histology laboratory, the slides are microscopically reviewed and a diagnosis rendered blindly (without knowledge of family).

The data presented are based upon the microscopic review of pulmonary tissue from mice of the 7 sub-families comprising this pedigreed BALB/c colony. Animals with lesions of pulmonary emphysema were classified, using a scale of severity of lesions, from negative to 4+, based on a subjective rating system devised by Dr. Rabstein. Her criteria for classification were:

Negative - no perceptible overdistention

1 plus - minimal overdistention in minimal number of lobes.

2 plus - slight overdistention in more than 1 lobe.

3 plus - mild to moderate overdistention in 1 or more lobes.

4 plus - areas of severe overdistention and/or bullae formation in one or more lobes.

Fig. 1 illustrates representative examples of each classification.

In 1973 Dr. Rabstein reported familial variance of spontaneously occurring generalized emphysema in the old BALB/c mice (greater than 18 months)*. Dr. Robert Kovatch and other consultant pathologists reviewed and concurred in these findings. The histomorphology of the emphysema in mice fits most nearly into the panacinar (diffuse vesicular) or alveolar category. The more prominent lesions in BALB/c mice occur toward the periphery of the lung lobes. Centrilobular emphysema as described in human lungs has not been observed.

Large numbers of mice have been studied since that time by Dr. Bernard Sass, and the differing familial incidences have been reconfirmed, lending credence to the possibility of developing a congenital emphysema model for experimental research purposes.

* Report, L. Rabstein to J. Kreisher, C.T.R., Feb. 1973.

METHODS

Gross and Microscopic Examinations

Mice are sacrificed by placing them in a chamber containing dry ice. When anaesthetized, they are killed by exsanguination from the brachial artery. The heart and lungs are removed from the thoracic cavity in toto. A complete gross examination is also performed. Animals whose lungs are to be inflated are handled as above, but in addition, approximately 1.5 cc of 10% neutral buffered formalin is instilled intratracheally at a pressure of approximately 10 cm water prior to removal from the thoracic cavity. A 100 ml burette is used as a reservoir and plastic tubing with an attached blunt 18 ga needle serves as a canula. The trachea is clamped off and tied following perfusion. The heart and lungs are then immersed in 10% neutral formalin overnight. The entire heart and lungs are processed, embedded in paraffin and 6 micron sections are cut and stained with Hematoxylin and Eosin.

$Alpha_1$ Antitrypsin

Blood samples are collected by the orbital bleeding technique using .280 ml capillary tubes. When the blood is clotted, the tubes are centrifuged at 3000 rpm for 15 minutes in a refrigerated centrifuge. The resultant serum is frozen and submitted to Dr. D. Michaeli, Dept. of Biochemistry, University of California Medical School, San Francisco. The method of analysis for $alpha_1$ antitrypsin is the enzymatic method of Sachar *et al* (1955).

Physiology

Functional residual capacity will be measured in the anaesthetized mouse by the method of Watanabe and Aviado (in press). Tidal volume, pulmonary resistance and pulmonary compliance measurements will be obtained using methods outlined by Policek et al (1967) and Ito and Aviado (1968).

RESULTS TO DATE

Incidence and Severity

Table A-1 is a tabulation of the incidence and severity of emphysema in 567 individual mice from the 7 sub-families. The percentage of individuals with 3+ or 4+ emphysema ranged from a low of 38% for Family 1 to a high of 61% for Family G. The average of all families was 52%. Table A-2 shows the mean age of mice in each family with the different degrees of severity of pulmonary emphysema.

On the basis of these findings, Families S, X and Y were excluded from further analysis of results, leaving 3 families (G, L and 2) with a higher than average incidence of severe (3+ or 4+) emphysema and one (Family 1) with a lower than average incidence. Family 1 was retained as a low incidence control sub-strain. The comparative difference in incidence and severity of emphysema between the high and low incidence families is shown in Fig. 2. There is more of the severe (3+ and 4+) emphysema in the high incidence families. In Family 1 (low incidence), the trend is toward the milder forms of emphysema.

Age Relationships

Figure 3 and Table A-2 shows that the severity of emphysema, in general, increases with advancing age. Further, the mean age of occurrence of emphysema is generally earlier in the high incidence families.

Sex Differences

The incidence and severity of emphysema by sex was determined and is shown in Table B and Fig. 4. This was done because more female mice were kept to old age. Emphysema of all levels of severity was generally equal in distribution between the two sexes.

Effect of Method of Fixation

Early in this study, the degree of emphysema was established based on uninflated, formalin-fixed lung sections. Later, it was suggested the accuracy of diagnosis would be improved if the lungs were fixed in an inflated state. After that time, most of the lungs were inflated. Table C is a tabulation of the incidence and severity of emphysema in inflated and uninflated lungs. The comparison is summarized in Fig. 5. The incidence of all degrees of severity of emphysema in uninflated lungs is comparable with that of inflated lungs. This suggests that, although there may be an advantage to examining the inflated specimen, the data from uninflated lungs is acceptable for computations of incidence.

Age Association

The frequency of emphysema in mice of various ages is shown in Table D. Frequency was calculated for negative, 1+ and 2+ and for 3+ and 4+. The ages of greatest incidence for the negative, 1+ and 2+ group was from 16 through 24 months of age (79% of individuals).

The 3+ and 4+ group was 19 through 27 months of age (84% of individuals). In both groups, over 60% were in the 19 through 24 month age group. This indicates that 3+ or 4+ emphysema is infrequently found in this strain of mice prior to 18 months of age.

The incidence of severe (3+ or 4+) emphysema in the high (Families G, L and 2) and low (Family 1) incidence families, at various age groups, is compared in Table E and Fig. 6. Except for the young and the very old age groups, where numbers of observations are too small to be valid, the frequency of occurrence of severe emphysema is markedly greater in the high incidence families, involving over 50% of the mice examined at ages above 19 months.

Associated Lung Disease Incidence

Table F, which lists the frequency of observance of lesions other than emphysema, indicates that the age at which emphysema is most often seen coincides with the age of greatest frequency of other findings. The frequency of findings of the various categories of pulmonary and non pulmonary lesions is shown in Table G.

The data in Tables F and G for the high incidence families (G, L and 2) were combined and compared to the low family, based on the numbers of mice with lesions divided by the total number observed for each age group. The results are seen in Table H and Figures 7 and 8.

For pulmonary lesions (Fig. 7) the high incidence families had greater frequency at all age groups except the 19-21 month group, where the frequency is equal. The frequency of non-pulmonary lesions (Fig. 8) was more nearly equal in the two groups for those ages (16-24 months) in which there were significant numbers of mice to compare.

The comparative frequency of the various types of lung lesions in the 4 families (Fig. 9) indicates that Family 1 had a small but consistently lower frequency of lesions of all types except reticuloendothelial neoplasms. Here, the incidence was essentially the same for all families. Family L, a high incidence family, had frequencies for all lesions similar to low incidence family 1. This interesting observation will be closely followed in continuing genetic studies.

Table I demonstrates the general comparability of the four families when the 3+ and 4+ emphysema is matched by age with other pulmonary and non pulmonary lesions. This emphasizes that, in general, all families had the same experience; that of having emphysema at approximately the same age as other lesions. These data suggest that the emphysema may well be related to the occurrence of other pulmonary lesions as well as space-taking lesions of other organ systems. The fact that, in spite of this,

there are families characterized by low and high emphysema incidence, argues for factors other than concurrent lesions having a part in the frequency of occurrence of emphysema.

Table J is a compilation, by family, of those mice with various pulmonary lesions and associated mild (negative to 2+) or severe (3+ and 4+) emphysema. Based on a very small sample, the severe emphysema is, in general, more frequently seen in animals with other pulmonary lesions than is the mild emphysema. Family 1 is the exception; lung tumors and other lung lesions were approximately the same in the neg to 2+ and the 3+ and 4+ groups. Family 2 had the highest incidence of concurrent lung lesions with 3+ and 4+ emphysema as compared with the neg to 2+ incidence. (Family G, 2:1; Family L, 2:1; Family 2, 9:1; Family 1, 1:1.)

The comparative frequency of severe emphysema with concurrent lung lesions in the high and low incidence families is seen in Fig. 10. Although based on small numbers of animals for each age group in Family 1, there is a consistent pattern of greater frequency in the high incidence families. As with the other tabulations, the increase in occurrence with increasing age is obvious for both groups.

Alpha$_1$ Trypsin Inhibition Assay

In collaboration with Dr. Dov Michaeli, of the University of California at San Francisco, preliminary studies of alpha$_1$ antitrypsin levels have been carried out on young mice being held for future disease studies. Individual and sex associated differences were observed in the various families. Males had significantly higher alpha$_1$ trypsin inhibitor as is shown in Fig. 11. In initial studies of a limited number of emphysematous mice significant blood hemolysis occurred, but no correlation was evident between percentage of inhibition and emphysema expression. This alpha$_1$ trypsin inhibitor series is being repeated in a larger number of animals.

Pedigree Definition

When individual mice with 3+ or 4+ emphysema are identified on the pedigree chart of a family, it becomes possible to separate, within the family, those breeding lines which have the greatest or least numbers of individuals with emphysema (Fig. 12).

Mice from this pedigreed BALB/c colony having 3+ and 4+ emphysema, reflect an average incidence of 52% at 21 months of age, with highest incidence (61%) in Family G. Family 1, at the other extreme, had an incidence of 38% (Table A). This suggests there might be a genetic determinant of predisposition for emphysema. The fact that the incidence of concurrent pulmonary and non-pulmonary pathologic lesions displays less than the above variation between the high and low incidence families (Table F) further suggests a genetic factor.

DISCUSSION OF RESULTS TO DATE

The mice from which these data were developed were kept for another purpose; the definition of the natural history of neoplasia throughout their life span. This precluded doing serial sacrifices and leaves unanswered the question of the rate at which emphysema develops, the progression of the lesion and the earliest age at which a 50% incidence of emphysema can be predicted. It also precluded concurrent physiological and biochemical studies such as are included in the following proposal. Studies of this nature would require establishment of a colony specifically for utilization by CTR, with the concurrent investigation of specifically associated causative factors.

EXPERIMENTAL DESIGN AND PROCEDURES

Breeding and Holding Colony

Microbiological Associates proposes to initiate a two part program to define the genetics of emphysema in mice. First a production (breeding) colony of pedigreed BALB/c mice will be maintained, utilizing breeders selected from those parental lines in which the expression of emphysema is high or low. Second a holding (research) colony for studying age at incidence of emphysema onset and for physiology, biochemical and immunological testing in the three high incidence pulmonary emphysema families and the one low incidence family.

The production will be based on the numbers of breeding and holding mice that can be maintained on 15 mouse cage racks. It is estimated that this will,in time, provide a constant yield of approximately 100 mice, 24 month old, per month. Actual numbers at each age increment will depend upon the number of mice sacrificed for experimental studies prior to 24 months of age.

Four pedigree families; one low incidence line (Family 1) and 3 high incidence lines (Families G, L and 2) will be maintained. Accurate pedigrees will be kept on each family, to include positive identification of all breeder mice by ear and/or toe marking. Holding colony mice will be separated by sex and each age will be identified by cage

cards. Each mouse will be identified by its sub-family and parents. At the end of their active breeding life, the production colony mice will be added to the holding colony population. This will provide an added number of mice for experimental purposes.

Once the holding colony is established, breeding schedules will be set to assure a steady number of weanling age mice. Continued accumulation of data on which to base the breeding program will be carried out during the transfer of effort from NCI contract #NCI CP-33248 to CTR. In the second year, mice 12 months of age will become available for sampling to determine pulmonary physiological and biochemical parameters.

Sampling for serologic determination of the murine virus profile of the colony will be scheduled at regular intervals, to assure that the disease status of the colony is always known. There will be a similar bacteriological monitoring of the mice.

When a definitive baseline of physiological, biochemical and pathological parameters has been established, it will become possible to conduct induction studies utilizing a variety of materials. Such induction studies might well lead to the changing of the panlobular emphysema to the centrilobular type most frequently seen in humans.

As it becomes possible to predict which individual mice will develop severe emphysema, more definitive genetic studies can be planned, utilizing f_1 hybrids and back crosses to identify gene loci of the hereditary factors contributory to the development of severe emphysema.

LUNG PHYSIOLOGY, BIOCHEMISTY AND PATHOLOGY

A collaborative study with consultant Dr. D. Aviado of the Dept. of Pharmocology, Univ. of Penna. will be undertaken to define those physiological parameters which might be predictive of the disease status of living mice. Groups of animals will be withdrawn from the holding colony at different ages for study. Male and female mice from each family will be killed at 3 month intervals to establish a histologic diagnosis of emphysema. The parameters of α_1 antitrypsin inhibitor level, specific serum lung collogen antibodies, functional residual capacity, pulmonary resistance and compliance, ventilatory response and blood gas analyses will be determined at regular 3 mo. intervals prior to sacrifice.

All animals on this Project that are sacrificed or die will be subjected to a complete gross and microscopic pathology examination. Results of this examination will be recorded and entered into a computerized storage. This will permit correlation of data with experimental results, physiological and biochemical testing procedures, and other ancillary data. Programs will be formulated to permit rapid retrieval and analysis of data to provide those correlations required to interpret the results of experimental protocols. The data input will include pedigree records to assist in establishing the genetic history of individual mice.

FACILITIES

The room to be set aside for this project is located in Bldg. #3, Ballenger Creek. It will provide a separate room within a disease barrier sustained colony building. This room meets or exceeds the requirements of the Guide for the Care and Use of Laboratory Animals, DHEW Publication No. (NIH) 73-23, revised 1972. The space is also accredited by the American Association for the Accreditation of Laboratory Animal Care. This gives maximum assurance against the inadvertent introduction of disease into the colony.

A temperature of $74^{o} \pm 4^{o}F$ will be maintained in the animal room. There will be 8-10 changes of air per hour with 100% air exchange (no recirculation).

Drinking water will be chlorinated to 10-12 PPM as an adjunct to control of Salmonella. All feed entering the building will be pasteurized and all bedding sterilized by steam autoclave.

Cage units (cage, lid and watering device) will be changed weekly. Before re-use they will be sanitized by passage through a 3 cycle cage wash machine. Cage wastes will be sealed in plastic bags for removal from the animal room.

Personnel assigned as animal caretakers will be utilized exclusively for this project. They will pass through a personnel entry lock each time they enter the

building. Here, they will remove their street clothes, shower, then change into a clean working uniform. This will include shoes to be worn only in the animal room, a disposable face mask and a disposable head cover.

REFERENCES

1. Thurlbeck, W.M., et al. Chronic Obstructive Lung Disease. Med. 49:81-145 (1970).
2. Snider, G.L., Hayes, J.A., Karthy, A.L. & Lewis, G.P. Centrilobular emphysema experimentally induced by cadmium chloride aerosol. Amer. Rev. Resp. Disease 108:40-48 (1973).
3. Krehl, V.E. The experimental production of pulmonary emphysema, A preliminary report. Amer. Rev. Resp. Disease 80:158-167 (1959).
4. Tura, S. Pulmonary emphysema and polycythemia induced in rats by forced swimming. Proc. Soc. Exp. Biol. Med. 103:713-715 (1960).
5. Gross, P., et al. Experimental emphysema: Its production with papain in normal and silicotic rats. Arch. Environ. Health 11:50-58 (1965).
6. Giles, R.E. Production of emphysema like conditions in rats by administration of papain aerosol. Proc. Soc. Expt. Biol. & Med. 134:157-162 (1970).
7. Pugh, P.S. Greyhound Racing Association (personal communication).
8. Eyre, P. Equine Pulmonary Emphysema - A Bronchopulmonary Mould Allergy. Vet. Rec. 91:134-140 (Aug. 1972).
9. Carlson, J.R., et al. Induction of pulmonary edema and emphysema in cattle and goats with 3-methylindole. Science 176:298-299 (21 Apr 1972).

10. Nettesheim, P., (personal communication).

11. Freeman, G., et al. Subacute NO_2 induced lesions of rat lung. Arch. of Env. Health (Chicago) 18:609-612 (April 1969).

12. Goldring, I.P., et al. Pulmonary effects of SO_2 in hamsters II. Combinations with emphysema. Arch. of Env. Health (Chicago) 21:32-37 (June 1970).

13. Eriksson, S. Pulmonary emphysema and $Alpha_1$ antigen defining. Acta Med. Scand. 175:197-205 (1964).

14. Chan, S.K. $Alpha_1$ antitrypsin in sera of inbred mice. Arch. Environ. Health. 27:271-272 (Oct. 1973).

15. Sachar, L., et al. An enzymatic method for the determination of $Alpha_1$ antitrypsin. Proc. Soc. Exptl. Biol. and Med. 90:327 (1955).

16. Aviado, D.M. and Watanabe, T. Functional and Biochemical effects on the lung following inhalation of cigarette smoke. I-High and low nicotine cigarettes in mice. Submitted to Toxicol. Appl. Pharmacol.

17. Palecek, F., et al. Emphysema in immature rats - a condition produced by tracheal constriction and papain. Arch. Env. Health 15:332-342, (1967).

18. Ito, H. and Aviado, D. Pulmonary emphysema and cigarette smoke - Experimental induction and use of Bronchodilators in rats. Arch. Env. Health 16:865-870.

BUDGET - FIRST YEAR

A.	Direct Labor (Schedule A)	$22,770
B.	Overhead (115% of A)	26,186
C.	Other Direct Costs (Schedule B)	21,500
D.	Travel	1,500
E.	Total Before G & A	71,956
F.	General and Administrative (16% of E)	11,513
G.	Overtime Premium	48
H.	Total Cost	83,517
I.	Fixed Fee	9,283
J.	Total Before Equipment & Facilities Rearrangement	92,800
K.	Facilities Rearrangements	5,000
L.	Equipment (Schedule C)	20,000
M.	Total Price	$117,800

SCHEDULE A

DIRECT LABOR:

Name	Function	Time on Project	Total Hours*	Hour $
R. Nims	Project Director	15%	289	REDACTED
B. Sass	Veterinary Pathologist	10%	193	REDACTED
B. Getzandanner	Histology Technician	10%	193	REDACTED
W. Athey	Animal Technologist	10%	193	REDACTED
Vacancy (to start last 3rd of year)	Technician	100%	642	REDACTED
J. Disney(to start 4th quarter)	Animal Caretaker	100%	482	REDACTED
P. Lee	Animal Caretaker	100%	1,926	REDACTED
M. Haven	Computer Programmer	10%	193	REDACTED
P. Gradwell	Research Clerk	10%	193	REDACTED

* Less 7.4%

Total Hours: 4,304

Total Direct Labor: 22,107

Plus 6% Merit Raise (3% for 6 months) 663

TOTAL DIRECT LABOR: $22,770

10035361Z4

CURRICULUM VITAE FOR PROFESSIONAL STAFF

A. Robert M. Nims, D.V.M.

B. Bernard Sass, M.S., V.M.D.

C. Wilbur L. Athey, B.S.

Soc. Sec. No. 448-03-2322

CURRICULUM VITAE - ROBERT M. NIMS

BIRTH: REDACTED

EDUCATION:

1956	Postgraduate Training (Surgery) School of Veterinary Medicine University of Pennsylvania, Philadelphia
1944	D.V.M., Iowa State University, Ames
1941	Pre-Veterinary Medicine Oklahoma State University, Stillwater

PROFESSIONAL AFFILIATIONS: REDACTED

PRESENT POSITION: 1970 - present REDACTED

POSITION DESCRIPTION: REDACTED

PRIOR EXPERIENCE:

1968 - 1970	REDACTED
1967 - 1968	REDACTED
1964 - 1967	REDACTED
1946 - 1963	
1944 - 1945	REDACTED

PUBLICATIONS - ROBERT M. NIMS

Glyceride Content of Human and Canine Red Blood Cells. Vacca, J.B., Waring, P.P., and Nims, R.M. Proceedings of the Society for Exp. Biol. and Med., 105: 100-102, 1960.

Human Body Volumeter Based on Water Displacement. Allen, T.H., Krzywicki, H.J., Worth, W.S., and Nims, R.M. U.S. Army Medical Research and Nutrition Laboratory Report 250, 1960.

Canine Mammary Adenocarcinoma with Metastasis to Bone. Nims, R.M., Dean, E.E., and Geil, R.G. A Case Report, Journal American Veterinary Med. Assoc., 138: 2: 87-89, 1961.

Studies in Segmental Replacement of the Thoracic Trachea. Aronstam, E. M., Nims, R.M., and Winn, D.F., Jr. Journal of Surgical Research, 1: 2: 108-110, 1961.

Fabrication of a Canine Respiratory Face Mask. Nims, R.M., and Worth, W.S. Jour. App. Physiol. 16: 1139-1140, 1961.

Embolectomy in the Dog. Nims, R.M. J.A.V.M.A., 140: 618-672, 1962.

Experimental Dislocation of the Femur, Delivered at Orthopedic Section Meeting, AMA, American Hotel, Miami Beach, Florida. Travis, L.O., Nims, R.M., Haupt, E.C., Omar, G.C., and Arnold, R.A., 1963.

Cervical Ganglioneuroma in a Dog. Ferrell, J.J., Hunt, R.D., and Nims, R.M., J.A.V.M.A., 144: 508-512, 1964.

Ehrlichia canis--The Causative Agent of a Hemorrhagic Disease of Dogs? Huxsoll, D.L., Hildebrandt, P.K., Nims, R.M., Ferguson, J.A., and Walker, J.S. Vet. Rec., 85: 587, 1969.

Clinical and Clinicopathologic Findings in Tropical Canine Pancytopenia. Walker, J.S., Rundquist, J.D., Taylor, R., Wilson, B.L., Andrews, M.R., Barck, J., Huxsoll, D.L., Hildebrandt, P.K., Hogge, A.L., and Nims, R. M. J.A.V.M.A., 157: 43-55, 1970.

Experimental Ehrlichiosis in Young Beagle Dogs. Hildebrandt, P.K., Huxsoll, D.L., and Nims, R.M. Fed. Proc., 29: 754, 1970 (Abstract).

The Pathology of Canine Tropical Pancytopenia. Hildebrandt, P.K., Huxsoll, D.L., Nims, R.M., and Walker, J.S. Lab. Invest., 22: 500, 1970 (Abstract).

Epizootiology of Tropical Canine Pancytopenia. Huxsoll, D.L., Hildebrandt, P.K., Nims, R.M., Amyx, H.L., and Ferguson, J.A. Journal of Wildlife Diseases, 6: 220-225, 1970.

Tropical Canine Pancytopenia.. Huxsoll, D.L., Hildebrandt, P.K., Nims, R. M., and Walkers, J.S. J.A.V.M.A., 157: 1627-1632, 1970.

PUBLICATIONS (Cont.)
R.M. NIMS

Epizootiology of Tropical Canine Pancytopenia in Southeast Asia. Nims, R.M., Ferguson, J.A., Walker, J.L., Hildebrandt, P.K., Huxsoll, D.L., Reardon, M.J., Varley, J.E., Kolaja, G.J., Watson, W.T., Shroyer, E. L., Elwell, P.A., Vacura, G.W. J.A.V.M.A., 158: 53-63, 1971.

Development of Hypergammaglobulinemia in Tropical Canine Pancytopenia. Burghen, G.A., Beisel, W.R., Walker, J.S., Nims, R.M., Huxsoll, D.L., and Hildebrandt, P.K. Am. J. Vet. Res., 32: 749-756, 1971.

Tropical Canine Pancytopenia. Huxsoll, D.L., Hildebrandt, P.K., Nims, R. M., and Walker, J.S. in Kirk: Current Veterinary Therapy-IV, Pub., Saunders Co., 677-679, 1971.

Laboratory Studies of Tropical Canine Pancytopenia. Huxsoll, D.L., Amyx, H. L., Hemelt, I.E., Hildebrandt, P.K., Nims, R.M., and Gochenour, W.S., Jr. Exper. Parasit, 31: 53-59, 1972.

An Improved Method for Enumeration of X-C Cell Assay for Murine Leukemia Virus. Spahn, G.J., Nims, R.M., Peters, R.L., and Kenyon, K. Applied Micro., 25: 149-150, 1973 (January).

Production of Hyperimmune Serum With Mature Rabbits. Nims, R.M., and Reeder, D.J. Lab. Animal Science, 23: 391-396, 1973 (June).

Pathology of Canine Ehrlichiosis (Tropical Canine Pancytopenia). Hildebrandt, P.K., Huxsoll, D.L., Walker, J.S., Nims, R.M., Taylor, R., and Andrews, M.A. Amer. Jour. Vet. Res. 34: 1309-1320, Oct. 1973.

CURRICULUM VITAE

NAME: Bernard (NMI) Sass

ADDRESS: REDACTED

PLACE OF BIRTH: REDACTED

DATE OF BIRTH:

EDUCATION:			academic degree
	New Brunswick High School		
	Rutgers University	1952-56	B.S.(Poultry Sci.)
	Univ. of Illinois	1956-57	Nutrition-Biochemistry research assistantship
	Univ. of Pennsylvania	1957-61	Doctor of Veterinary Medicine (V.M.D.)
	Univ. of Maryland	At present	M.S. Microbiology (should be received 1973)

MILITARY SERVICE: REDACTED

EXPERIENCE: 1963

1963-66 REDACTED

1966-1973 REDACTED

1974-present

PUBLICATIONS:

Messersmith, R.E., Sass, B., Berger, H., Gale, G.O. Safety and Tissue Residue Evaluations in Swine Fed Rations Containing Chlortetracycline, Sulfamethazine and Penicillin. JAVMA, 151, no. 6 (Sept. 15, 1967) pp 716-724.

Albert, T.F., McKinstry, D., Sass, B., Cason, J.L. Rectal Passage of a Duodenal Cannula in a Young Bull. Mod. Vet. Pract., Nov. 1968, p. 48.

10035361130

McKinstry, D.M., Sass, B., Cason, J.L. Albert T.F. Arteriosclerosis in Forestomach-Bypass Calves. J. of Dairy Sci., Vol. 52 No. 2 (Feb. 1969) pp. 273-276.

Mohanty S.B., Lillie, M.G., Albert, T.F. Sass. B. Experimental Exposure of Calves to a Bovine Rhinovirus. Am. J. Vet. Res., Vol. 30, no. 7 (July, 1969) pp. 1105-1111.

Sass, B., Albert, T.F. A case of Eisenmenger Complex in a Calf. Cornell Vet., Vol. LX, no. 1 (Jan. 1970).

Sass, B. Equine Strongylosis Threatens Horse Population. The Maryland Horse, Vol. 35, no. 4 (April, 1969) pp. 64-65.

Shillinger, R.B., Sass, B., Virts, H.A. An Apparent Outbreak of Botulism in Feedlot Cattle. Maryland Veterinarian, Vol. 12, no. 1 (Feb. 1970).

Sass, B. Perforating Gastric Ulcer Associated with Lead Poisoning in a Dog. JAVMA, Vol. 157, no. 1 (July 1, 1970) pp. 76-78.

Hemken, R.W., Vandersall, J.H., Sass, B., Hibbs, J.W. Goitrogenic Effects of a Corn Silage-Soybean Meal Supplemented Ration. J. of Dairy Science, Vol. 54, no. 1 (Jan., 1971) pp. 85-88.

Mallack, J., Sass, B., Ludlum, K.W. Dirofilaria immitis in Hunting Dogs from an Area in Maryland. JAVMA, Vol. 159, no. 2 (July 15, 1971) pp. 177-179.

Sass, B., Ludlam. K.W., Mallack, J. Response by Practicing Veterinarians to a Questionnaire on Dog Heartworm in Maryland. Southern Veterinarian, Vol. 9, no. 3 (May-June, 1972) pp. 14-15.

Sass, B. Hatziolos, B.C., Hayes, J.E. Probable Cadmium Poisoning in a Group of Ponies. Vet. Med., Vol. 67, no. 7 (July, 1972) pp. 745-746.

Sass, B. Bovine Herpes Virus DN 599--Characterization of the Agent by Immunofluorescence. M.S. Thesis, Graduate School, University of Maryland, College Park, Md.

Sass, B., Mohanty, S.B. and Hetrick, F.M. Bovine Herpes Virus DN599 in Characterization of the agent by Immunofluorescence. Am. J. of Veterinary Research.(In press)

McKinstrey, D.M., Carson, J.L., Albert, T.F. and Sass, B. Observations on the Health and Performance of Forestomach By-Pass and Control Calves Fed a Milk Replaced Diet - Submitted to Journal Airy Science.

CURRICULUM VITAE

NAME: Wilbur L. Athey

ADDRESS:

DATE OF BIRTH:

PLACE OF BIRTH: REDACTED

EDUCATION: High School - Flintstone High School, Flintstone, Md.
B.S. Agriculture - University of Maryland, College Park, Md. 1954

MILITARY SERVICE:

REDACTED

REDACTED

EXPERIENCE:

1942-1944 Operated 180 Acre General Farm
1946-1950

1954-1955

1955-1958 REDACTED

1958-1962

July 1962 - present REDACTED

ORGANIZATIONS:

REDACTED

TABLE A.

1. Incidence and Severity of Pulmonary Emphysema, by Family

Family	Severity of Emphysema												
	Neg.		1+		2+		3+		4+		Total	Combined 3 & 4+	
	No.[a]	%[b]	No.	%	No.	%	No.	%	No.	%		No.	%
G	2	1	8	8	28	29	42	44	17	18	97	59	61
L	2	2	7	9	26	32	36	44	10	12	81	46	57
S	3	2	15	12	36	29	49	40	20	16	123	69	56
X	2	5	0	-	21	51	12	29	6	15	41	18	44
Y	2	3	11	18	24	39	22	36	2	3	61	24	39
1	10	8	16	19	26	31	26	31	6	7	84	32	38
2	2	2	12	15	19	23	31	40	16	20	80	47	59
TOTAL	23	4	69	12	180	32	218	38	77	14	567	295	52

a - Number of mice in group.
b - % of total mice observed in each family.

2. Mean Age of Occurrence of Pulmonary Emphysema, by Family

Family	Severity of Emphysema					
	Neg.	1+	2+	3+	4+	Combined 3 & 4+
G	21.0[a]	19.3	20.8	21.7	20.0	21.4
L	8.5	19.3	19.7	20.6	21.0	20.7
S	18.7	23.9	20.3	21.7	21.8	21.7
X	14.5	-	19.9	19.5	19.7	19.6
Y	18.5	17.9	20.3	20.6	21.5	20.7
1	18.4	19.5	20.3	21.7	20.0	21.4
2	12.5	17.2	19.2	21.3	21.1	21.2
ALL FAMILIES	17.0	19.8	20.1	21.2	20.8	21.0

a - Mean age (Months) of all mice in group.

TABLE B.

Comparative Incidence and Severity of Emphysema in Male and Female Mice

MALES

Family	Not Rated		Neg.		1+		2+		3+		4+		Total Mice
	No.[a]	%[b]	No.	%	No.	%	No.	%	No.	%	No.	%	
G	0	-	0	-	2	6	8	26	15	48	6	19	31
L	5	13	2	5	1	3	9	24	18	47	3	8	38
2	1	3	0	-	8	22	12	32	11	30	5	14	37
1	4	11	5	14	5	14	10	28	11	31	1	3	36
TOTAL	10	7	7	5	16	11	39	27	55	39	15	11	142

FEMALES

G	3	4	2	3	6	9	19	28	27	40	11	16	68
L	1	2	0	-	6	12	17	35	18	37	7	14	49
2	3	6	2	4	4	8	7	15	21	44	11	23	48
1	1	2	3	6	10	20	16	32	15	30	5	10	50
TOTAL	8	4	7	3	26	12	59	27	81	38	34	16	215

a - Number of mice observed

b - Percent of total mice observed in each family

TABLE C.

Comparative Incidence and Severity of Emphysema in Inflated and Uninflated Mouse Lung Specimens

Family	Status	Not Rated		Neg.		1+		2+		3+		4+		Total Mice
		No.[a]	%[b]	No.	%	No.	%	No.	%	No.	%	No	%	
G	Inf.	1	4	0	-	3	13	6	25	10	42	4	17	24
	Uninf.	2	3	2	3	5	7	21	28	32	43	13	17	75
L	Inf.	0	-	0	-	1	6	6	33	10	56	1	6	18
	Uninf.	6	9	2	3	6	9	20	29	26	38	4	13	69
2	Inf.	2	7	1	4	7	25	3	11	10	36	5	18	28
	Uninf.	2	4	1	2	5	9	16	28	22	39	11	19	57
1	Inf.	0	-	1	5	4	18	7	32	9	41	1	5	22
	Uninf.	5	7	9	13	12	18	19	28	17	25	5	7	67
TOTAL	Inf.	3	3	2	2	15	16	22	24	39	42	11	12	92
	Uninf.	15	6	14	5	28	10	76	28	97	36	38	14	268

a - Number of mice observed

b - Percent of total mice observed in each family

TABLE D.

Frequency of Mild (Negative to 2+) and Severe (3+ and 4+) Emphysema in Various Age Groups of Mice

Mice with 1+, 2+ or no Emphysema

Family	Age (Months)																Total Mice
	<10		10-12		13-15		16-18		19-21		22-24		25-27		28 & >		
	No.[a]	%[b]	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
G	2	5	1	3	0	-	6	15	15	38	11	28	5	13	0	-	40
L	5	12	1	2	0	-	4	10	19	46	8	20	3	7	1	2	41
2	2	5	1	3	3	8	11	30	12	32	5	14	3	8	0	-	37
1	2	4	2	4	2	4	10	18	19	35	16	29	4	7	0	-	55
Total	11	6	5	3	5	3	31	18	65	38	40	23	15	9	1	1	173

Mice with 3+ and 4+ Emphysema

G	1	2	0	-	1	2	2	3	19	33	22	38	11	19	2	3	58
L	2	4	0	-	1	2	4	9	18	39	15	33	5	11	1	2	46
2	0	-	1	2	4	9	5	11	11	23	17	36	9	19	0	-	47
1	1	3	1	3	1	3	2	6	9	28	11	34	6	19	1	3	32
Total	4	2	2	1	7	4	13	7	57	31	65	36	31	17	4	2	183

a - Number of mice observed
b - Percent of total mice observed in each family

TABLE E.

Frequency of Severe (3+ and 4+) Emphysema in High and Low Incidence Families, by Age Group

Family	Age (Months)															
	< 10		10-12		13-15		16-18		19-21		22-24		25-27		≥ 28	
	Freq.[a]	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
G	1/3	33	0/1	-	1/1	100	2/8	25	19/34	56	22/33	67	11/16	69	2/2	100
L	2/7	29	0/1	-	1/1	100	4/8	50	18/37	49	15/23	65	5/8	63	1/2	50
2	0/2	-	1/2	50	4/7	57	5/16	31	11/23	48	17/22	77	9/12	75	0/0	-
Total Fam. G, L & 2) High Inc.)	3/12	25	1/4	25	6/9	67	11/32	34	48/94	51	54/78	69	25/36	69	3/4	75
Family 1 (Low Inc.)	1/3	33	1/3	33	1/3	33	2/12	17	9/28	32	11/27	41	6/10	60	1/1	100

a - $\frac{\text{\# Positive}}{\text{Total in Age Group}}$ for each age group

TABLE F.

Frequency of Pulmonary and Non-Pulmonary Lesions in Mice of Various Age Groups

Mice with Pulmonary Lesions

Family	Age (Months)																Total Mice
	<10		10-12		13-15		16-18		19-21		22-24		25-27		28 & >		
	No.[a]	%[b]	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
G	2	5	1	2	0	-	5	12	11	27	12	29	9	22	1	2	41
L	1	4	1	4	0	-	4	17	8	33	5	21	5	21	0	-	24
2	0	-	1	3	2	6	4	13	7	23	12	39	5	16	0	-	31
1	1	4	1	4	0	-	3	13	8	35	7	30	3	13	0	-	23
Total	4	3	4	3	2	2	16	13	34	29	36	30	22	18	1	1	119

Mice with Lesions of Other Systems

G	1	2	1	2	0	-	2	4	23	41	23	41	6	11	0	-	56
L	4	9	0	-	0	-	4	9	16	36	15	33	5	11	1	2	45
2	0	-	1	3	3	9	7	21	10	29	8	24	5	15	0	-	34
1	1	2	1	2	0	-	4	9	15	33	15	33	9	20	0	-	45
Total	6	3	3	2	3	2	17	9	64	36	61	34	25	14	1	1	180

a - Number of mice observed

b - Percent of total mice observed in each family

TABLE G.

Frequency of Pathologic Lesions in Mice of High and Low Incidence

Pulmonary Emphysema Families

Family	Not Rated & Neg.		PULMONARY LESIONS								NON-PULMONARY LESIONS								Total Mice
			Lung Tumors		Pneu.		Re Neoplasm (Lung)		Total Pulmonary		Re Neo Other Organs		Leukemia		Misc.[c] Lesions		Total Non-Pulmonary		
	No.[a]	%[b]	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
G	13	12	25	23	9	8	7	6	41	38	23	21	0	-	32	29	55	50	109
L	27	28	12	13	8	8	4	4	24	25	13	14	2	2	29	31	44	46	95
2	19	23	19	23	8	10	4	5	31	37	14	17	4	5	16	19	34	40	84
1	25	28	13	15	4	4	3	3	20	22	19	21	6	7	19	21	44	49	89

a - Number of mice observed

b - Percent of total mice observed in each family

c - Miscellaneous lesions are listed on the following page

footnote c, Table G - Definition of "miscellaneous lesions"

I. Neoplasms:
- A. - Adrenal
  1. Adrenal cortical adenomas
  2. Adrenal cortical carcinomas
  3. Pheochromocytomas
  4. Accessory adrenal gland
- B. - Ovary-uterus
  1. Cystic adenomatoid hyperplasia
  2. Hemangioendothelioma & hemangioma, uterus & oviduct
  3. Fibrosarcoma, uterus
  4. Leiomyosarcoma, uterus
  5. Adenocarc., ovary or uterus
  6. Theca granulosa cell tumor, ovary or uterus
  7. Fibroma, cervix
- C. - Testis
  1. Interstitial cell tumor
- D. - Digestive system
  1. Salivary glands
     a. myoepithelioma
  2. Liver
     a. hemangioendothelioma
- E. - Skin, subcutaneous and mucous
  1. Carcinomas
     a. vagina
     b. mammary glands
     c. ear canal
  2. Hemangioendothlelioma of subcutis
- F. - Reticuloendothelial system
  1. Spleen
     a. hemangioendotheliomas

II. A. Abcesses
- B. Generalized infections
- C. Granulomas
- D. Cardiac valvular stenosis

III. Degenerative
- A. Liver necrosis
- B. Nodular hyperplasia of liver

IV. Physiological alterations
- A. Hyperplasia
  a. erythroid of spleen and lymph nodes
- B. Siderosis
  a. lung

TABLE H.

Frequency of Severe (3+ and 4+) Emphysema and Other Pathologic Lesions, By Age Group, in Families with High or Low Incidence of Pulmonary Emphysema

Characteristic	Incidence	AGE (Months) ≤10 Freq.[c]	%	10-12 Freq.	%	13-15 Freq.	%	16-18 Freq	%	19-21 Freq.	%	22-24 Freq.	%	25-27 Freq.	%	≥28 Freq.	%	TOTAL Freq.	%
Mice with Severe (3+ & 4+) Emphysema	High[a]	3/12	25	1/4	25	6/9	67	11/32	34	48/94	51	54/78	69	25/36	69	3/5	60	151/270	56
	Low[b]	0/3	-	1/3	33	0/3	-	2/12	17	9/28	32	11/27	41	6/10	60	1/1	100	32/87	37
Mice with Pulmonary Lesions	High	3/12	25	3/4	75	2/9	22	13/32	41	26/94	28	29/78	37	19/36	53	1/5	20	96/270	36
	Low	1/3	33	1/3	33	0/3	-	3/12	25	8/28	29	7/27	26	3/10	30	0/1	-	23/87	26
Mice with Non-Pulmonary Lesions	High	5/12	42	2/4	50	3/9	33	13/32	41	49/94	52	46/78	59	16/36	44	1/5	20	135/270	50
	Low	1/3	33	1/3	33	0/3	-	4/12	33	15/28	54	15/27	56	9/10	90	0/1	-	45/87	52
Mice c Severe Emphysema and Concurrent Pulm. Lesions	High	2/12	17	1/4	25	1/9	11	7/32	22	18/94	19	24/78	31	14/36	39	1/5	20	68/270	25
	Low	0/3	-	1/3	33	0/3	-	1/12	8	2/28	7	3/27	11	3/10	30	0/1	-	10/87	12

a - Total of High Incidence Families G, L and 2

b - Family 1 (Low Incidence)

c - $\left(\frac{\text{\# with lesion}}{\text{Total observed}}\right)$ at each age group

TABLE I.

Comparison of Pathologic Findings by Family, at Various Ages

Pathology	AGE (Months)																Total Mice
	<10		10-12		13-15		16-18		19-21		22-24		25-27		28&>		
	#[a]	%[b]	#	%	#	%	#	%	#	%	#	%	#	%	#	%	
FAMILY G																	
3+ or 4+ Emphysema	1	2	0	-	1	2	2	3	19	33	22	38	11	19	2	3	58
Pulmonary Lesions	2	5	1	2	0	-	5	12	11	27	12	29	9	22	1	2	41
Non-Pulmonary Lesions	1	2	1	2	0	-	2	4	23	41	23	41	6	11	0	-	56
FAMILY L																	
3+ or 4+ Emphysema	2	4	0	-	1	2	4	9	18	39	15	33	5	11	1	2	46
Pulmonary Lesions	1	4	1	4	0	-	4	17	8	33	5	21	5	21	0	-	24
Non-Pulmonary Lesions	4	9	0	-	0	-	4	9	16	36	15	33	5	11	1	2	45
FAMILY 2																	
3+ or 4+ Emphysema	0	-	1	2	4	9	5	11	11	23	17	26	9	11	0	-	47
Pulmonary Lesions	0	-	1	3	2	6	4	13	7	23	12	39	5	16	0	-	31
Non-Pulmonary Lesions	0	-	1	3	3	9	7	21	10	29	8	24	5	15	0	-	34
FAMILY 1																	
3+ or 4+ Emphysema	1	3	1	3	1	3	2	6	9	28	11	34	6	19	1	3	32
Pulmonary Lesions	1	4	1	4	0	-	3	13	8	35	7	30	3	13	0	-	23
Non-Pulmonary Lesions	1	2	1	2	0	-	4	9	15	33	15	33	9	20	0	-	45
TOTAL OF 4 FAMILIES																	
3+ or 4+ Emphysema	4	2	2	1	7	4	13	7	57	31	65	36	31	17	4	2	183
Pulmonary Lesions	4	3	4	3	2	2	16	13	34	29	36	30	22	18	1	1	119
Non-Pulmonary Lesions	6	3	3	2	3	2	17	9	64	36	61	34	25	14	1	1	180

a - Number of mice observed in each age group

b - Percent of total mice in each category

1003536142

TABLE J. Concurrent Findings of Emphysema with Other Pathology of the Pulmonary System.

	Family G			Family L			Family 2			Family 1		
	Severity		Total	Severity		Total	Severity		Total	Severity		Total
	Neg. to 2+	3+ & 4+		Neg. to 2+	3+ & 4+		Neg. to 2+	3+ & 4+		Neg. to 2+	3+ & 4+	
Alveolar Adenoma	9	8	17	2	6	8	1	7	8	6	4	10
Alveolar Adenocarcin.	1	7	8	1	3	4	1	10	11	0	3	3
Pneumonia	4	5	9	4	4	8	0	8	8	2	2	4
R.E. Neo.; Lung	0	7	7	1	3	4	1	3	4	1	2	3
All lung Lesions	14	27	41	8	16	24	3	28	31	9	11	20
All Tumors	10	22	32	4	12	16	3	20	23	7	9	16
Animals with Multiple Lesions			1			1			1			1

Text - Figure 1

Photomicrographs illustrating typical pathologic findings associated with each classification of pulmonary emphysema.

A - Negative - essentially normal - note intact alveolar walls. A slight degree of atelectasis is present.

B - 1+ - minimal overdistention of alveolar walls is present.

C - 2+ - slight overdistention of alveolar walls is present.

D - 3+ - mild to moderate overdistention of alveolar walls is seen. Also noted are focal areas of atelectasis.

E - 4+ - areas of severe overdistention of alveolar walls is present. Some atelectasis is noted.

F - 4+ - (Bullous emphysema) prominent areas of overdistention are seen beneath the pleura.

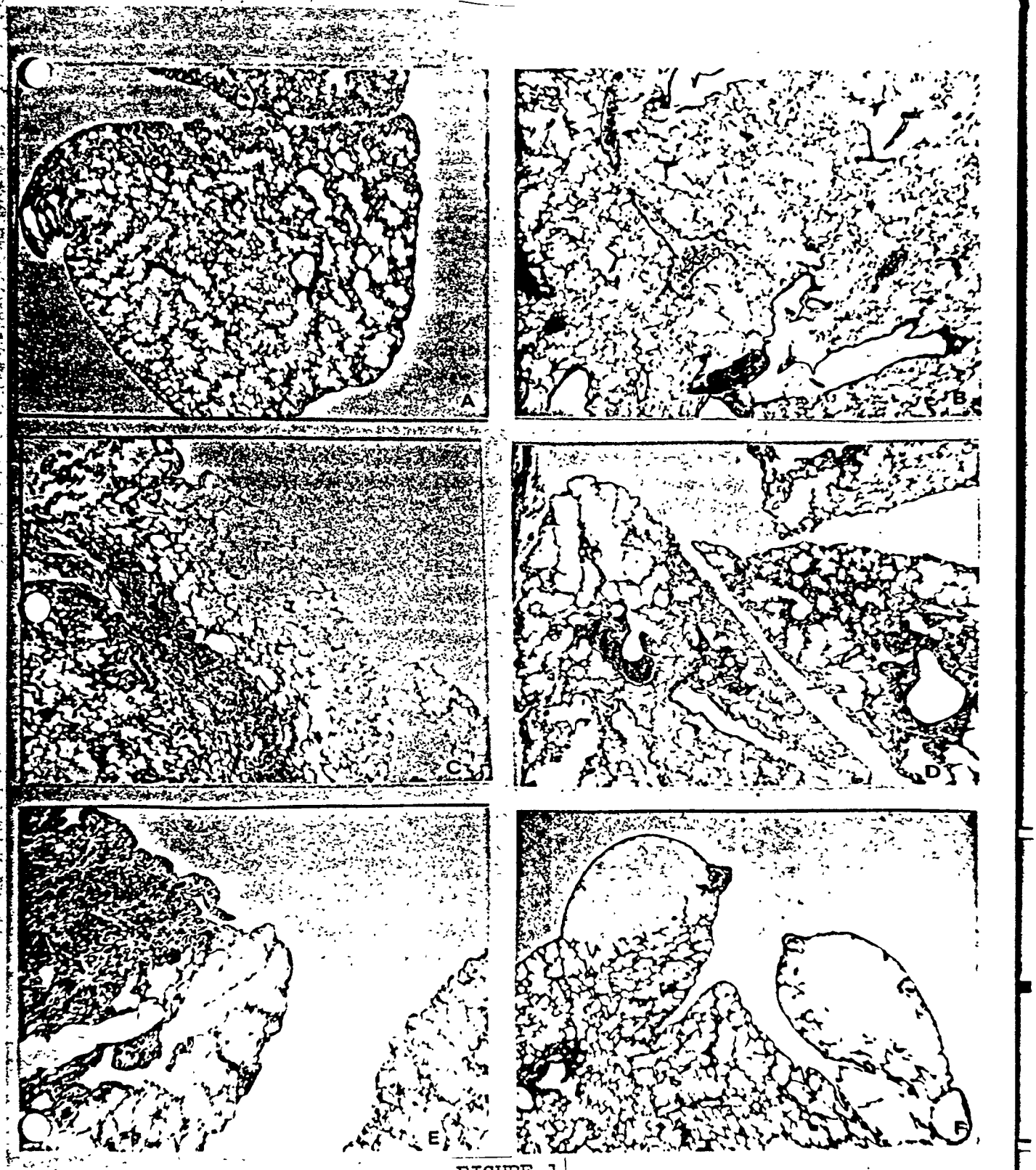


FIGURE 1

100536145

Text - Figure 2

Comparative incidence and severity of emphysema, High (Families G, L and 2) and Low (Family 1) Incidence Families.

The bars for High Incidence Families indicates the range of observations between the 3 Families. Each point is the $\left(\frac{\text{No. with emphysema}}{\text{Total population observed}}\right)$. The total number of mice in the High incidence families is 258; Family 1 is 84.

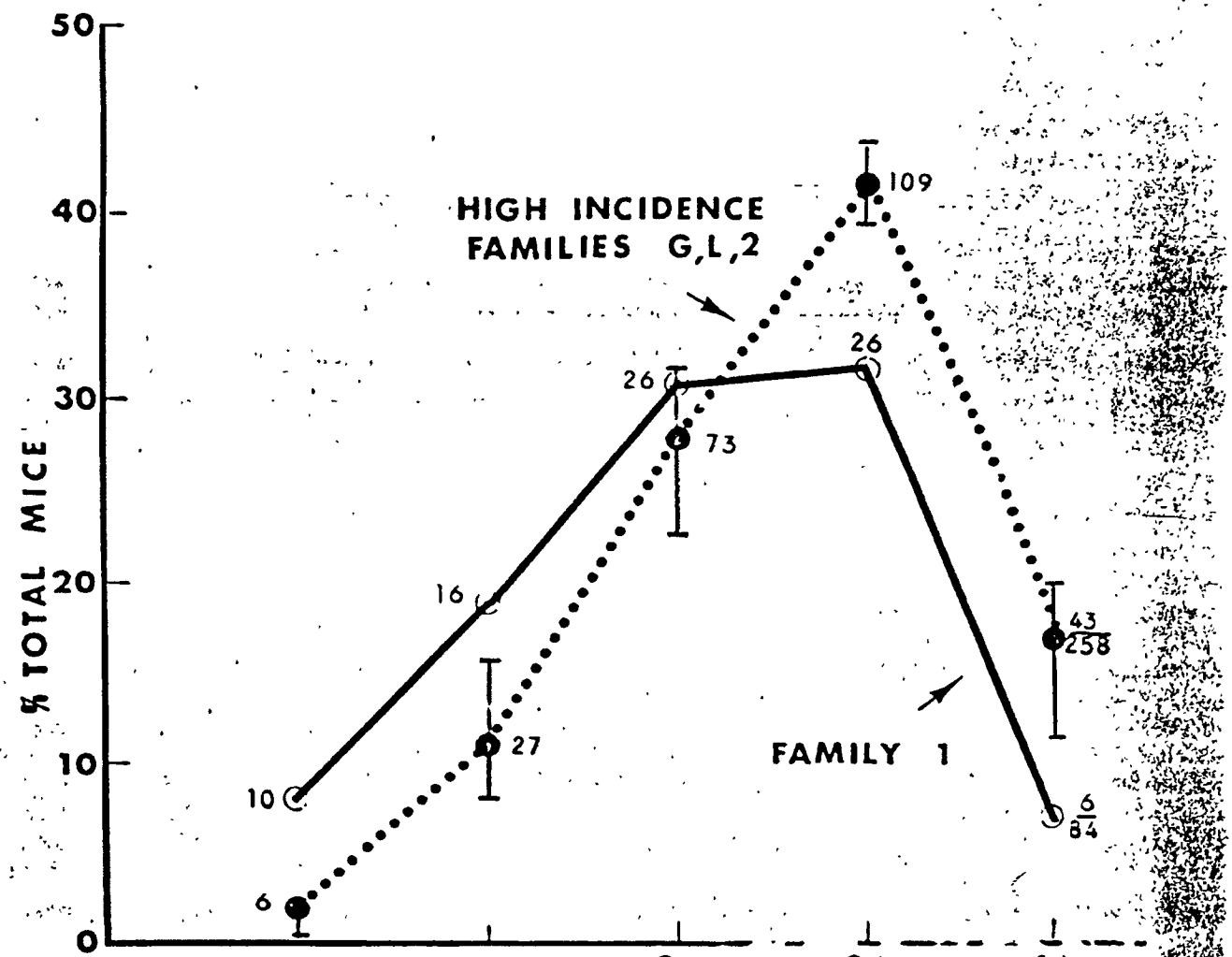


FIGURE 2

Text - Figure 3

Mean age of mice with each degree of emphysema in High (Families G, L and 2) and Low (Family 1) Incidence Families.

The bars for High Incidence Families indicates the range of observations between the 3 Families. Numbers beside the points indicate the number of mice comprising the group.

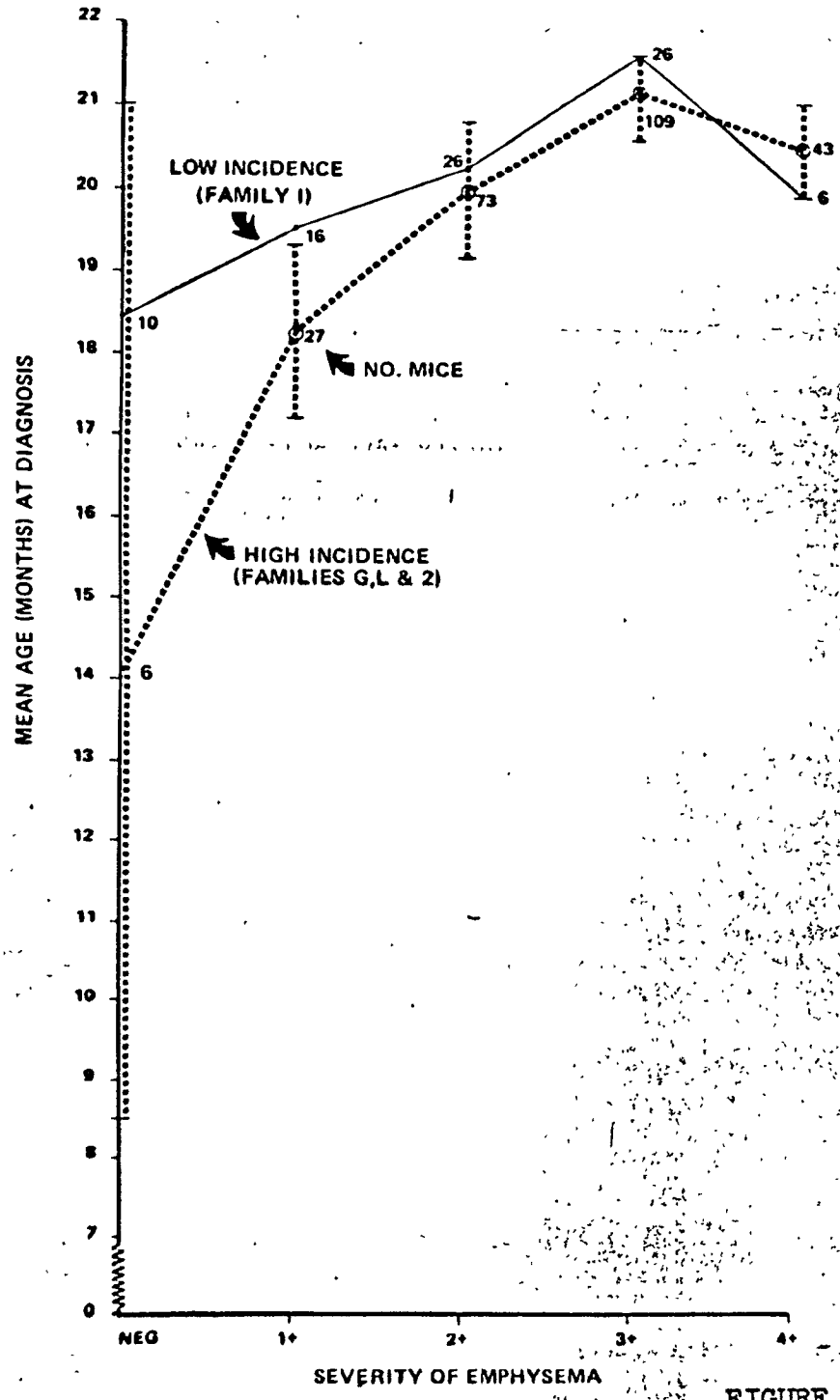


FIGURE 3

100536149

Text - Figure 4

Comparative Incidence and Severity of Emphysema between male and female mice.

The bars show the percent of total male or female mice with each severity of emphysema. The numbers at the base of each bar are the number of mice comprising the group.

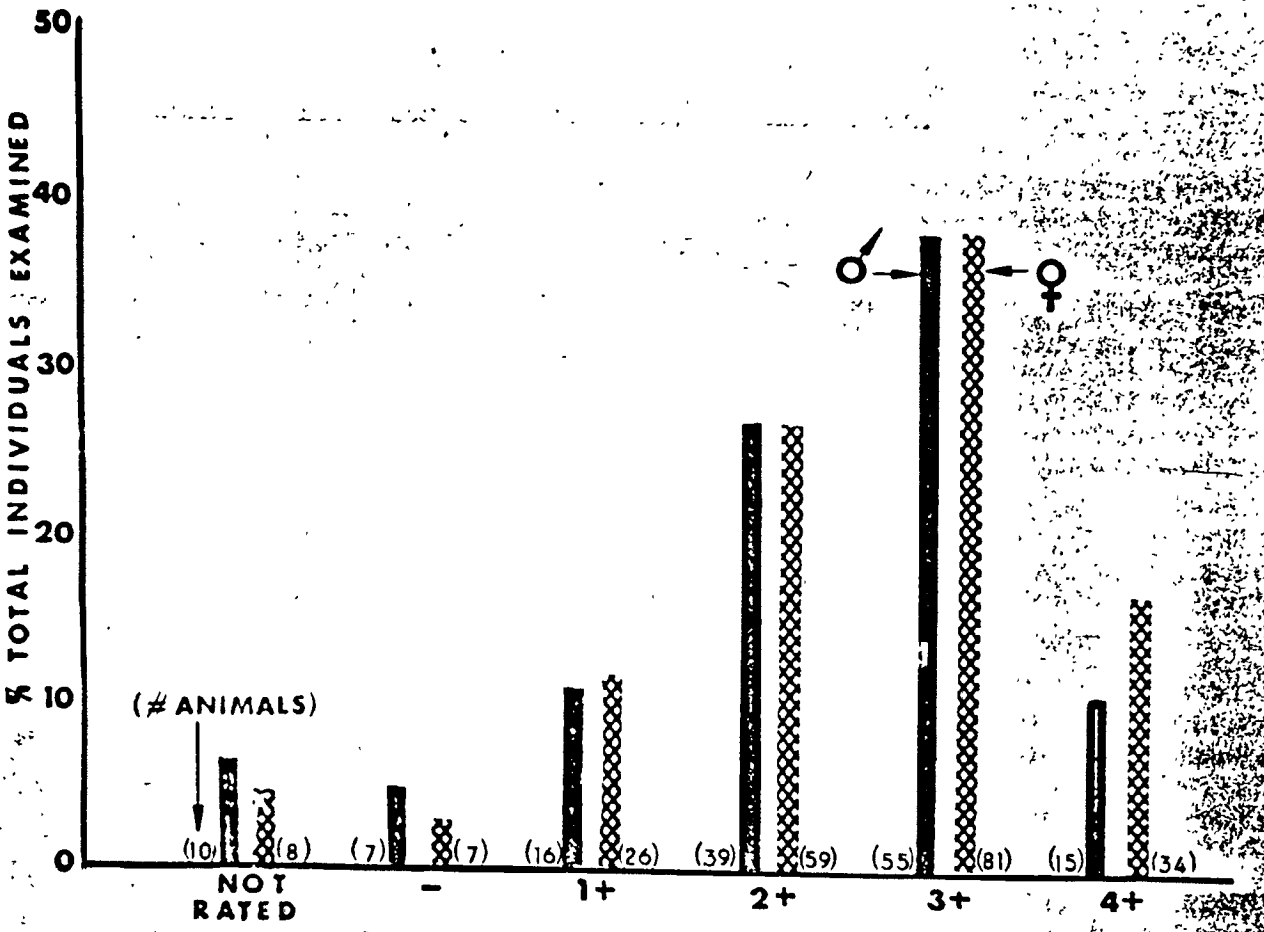


FIGURE 4

Text - Figure 5

Comparative Incidence and Severity of Emphysema in mice between formalin-fixed inflated or uninflated lung sections.

The bars show the percent of total inflated or uninflated mouse lung specimens examined with each severity of emphysema. The numbers at the base of each bar are the numbers of specimens comprising the group. There was a total of 92 inflated specimens; 268 uninflated.

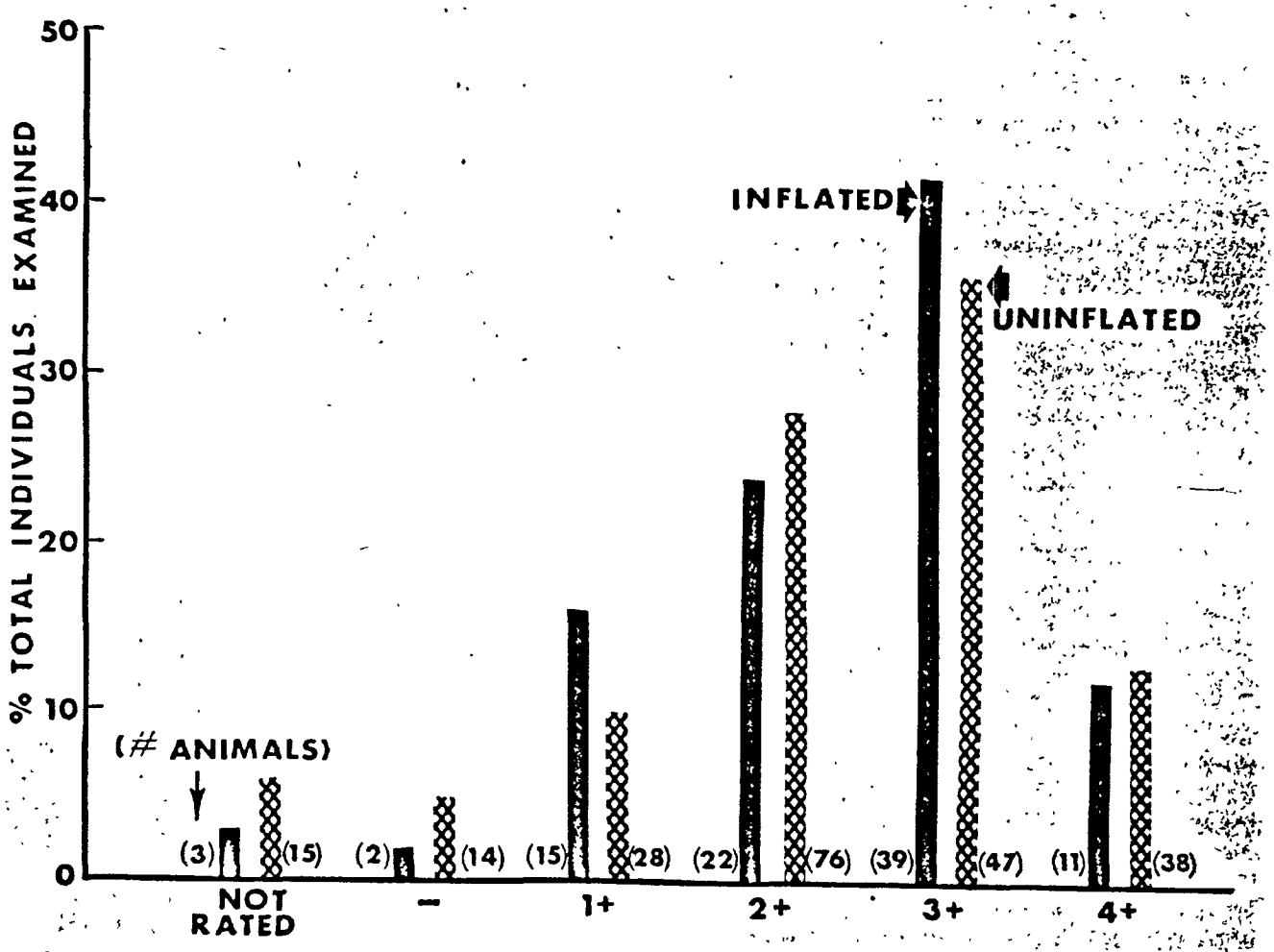


FIGURE 5

10035361 53

Text - Figure 6

Comparative Incidence of Severe (3+ or 4+) Pulmonary Emphysema, by age group, between High (Families G, L, and 2) and Low (Family 1) Incidence Families.

The number at each point is the $\left(\frac{\text{No. with severe emphysema}}{\text{Total observed}}\right)$ for each age group. Each age group spans a time of 3 months. The bars for High Incidence Families indicates the range of observations between the 3 families.

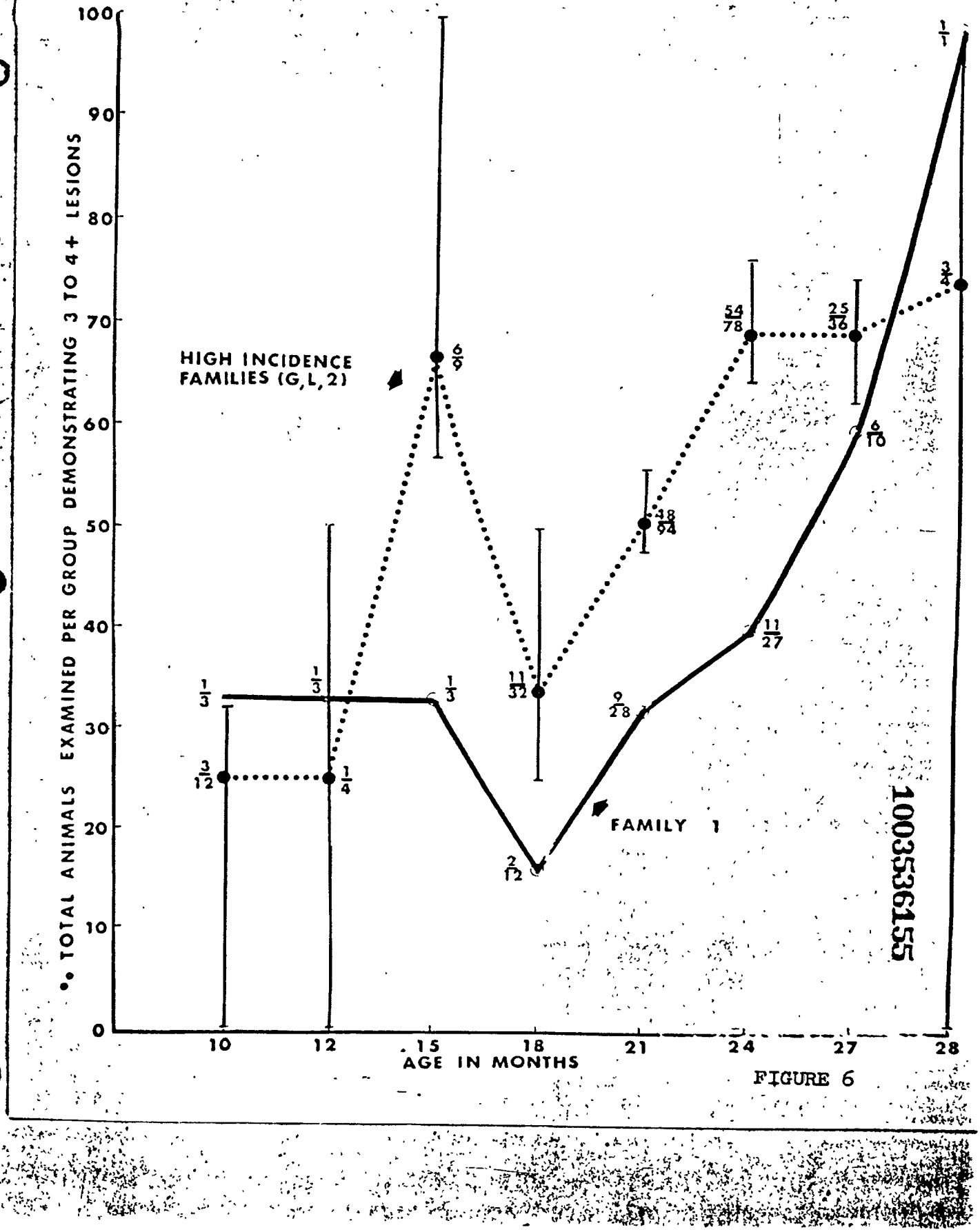


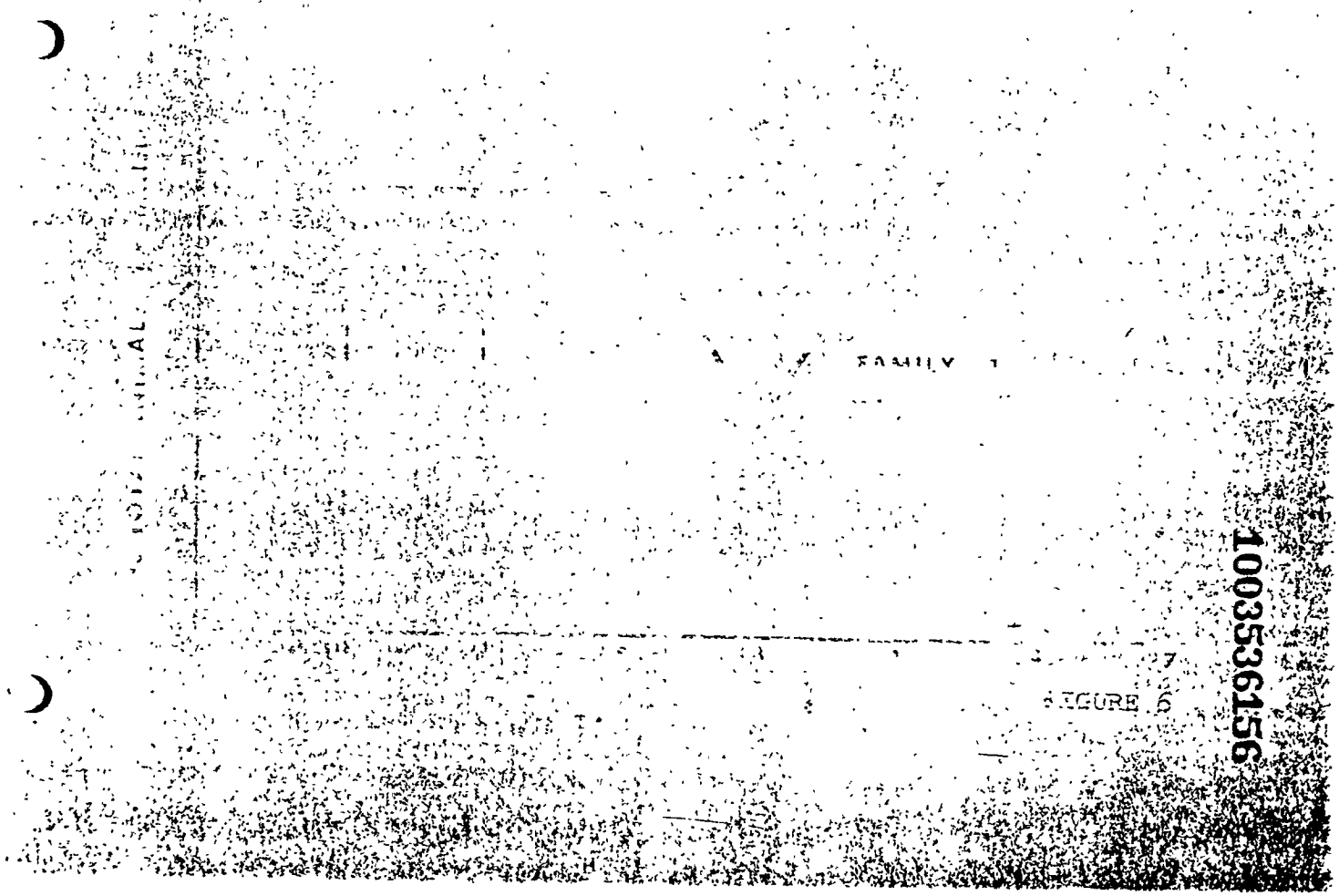
FIGURE 6

100353615S

Text - Figure 7

Comparative frequency of pulmonary lesions, by age group, in High (Families G, L and 2) and Low (Family 1) Incidence Families.

The number at each point is the $\left(\frac{\text{number positive}}{\text{total observed}}\right)$ for each age group. Each age group spans a time of 3 months.



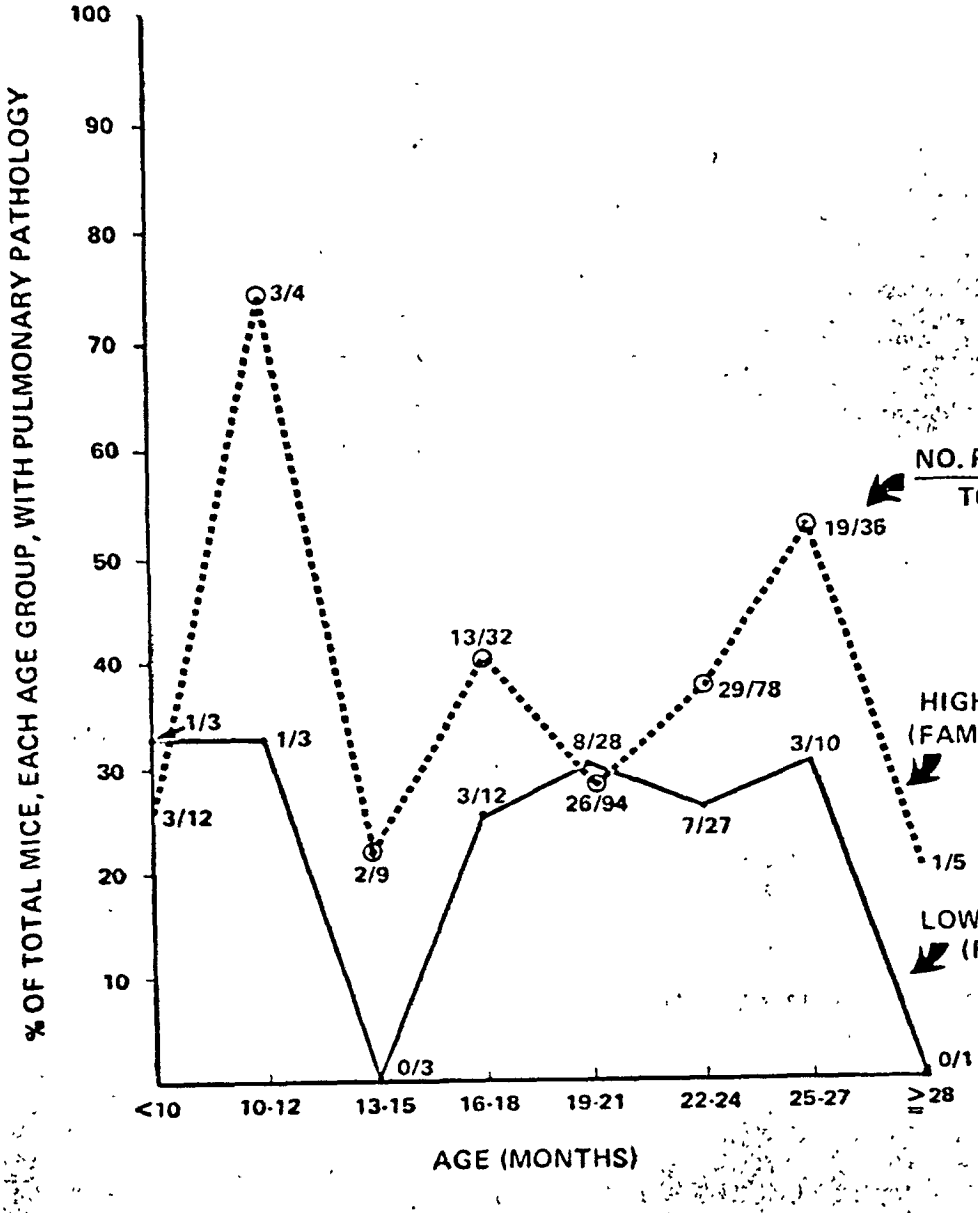


FIGURE 7

100353617

Text - Figure 8

Comparative frequency of non-pulmonary lesions, by age group, in High (Families G, L and 2) and Low (Family 1) Incidence Families.

The number at each point is the $\left(\frac{\text{number positive}}{\text{total observed}}\right)$ for each age group. Each age group spans a time of 3 months.

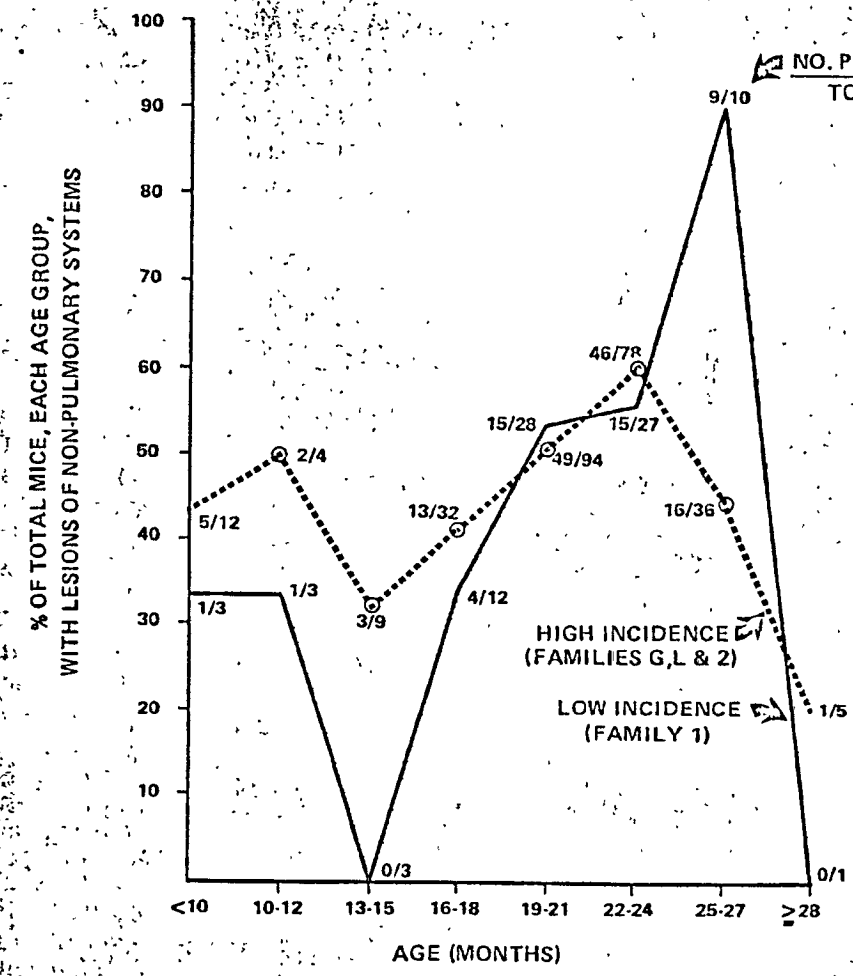


FIGURE 8

Text - Figure 9

Comparative frequency of occurrance of different type pulmonary lesions, by age group, in the High (Families G, L and 2) and Low (Family 1) Pulmonary Emphysema Incidence Families.

The % frequency represents the $\left(\frac{\text{number with lesion}}{\text{total observed}}\right)$ for each family. The number at the base of each bar indicates number of mice comprising the group. The average of the High Incidence Families (G, L and 2) is shown as a separate bar. Multiple lesions were found in some of the mice.

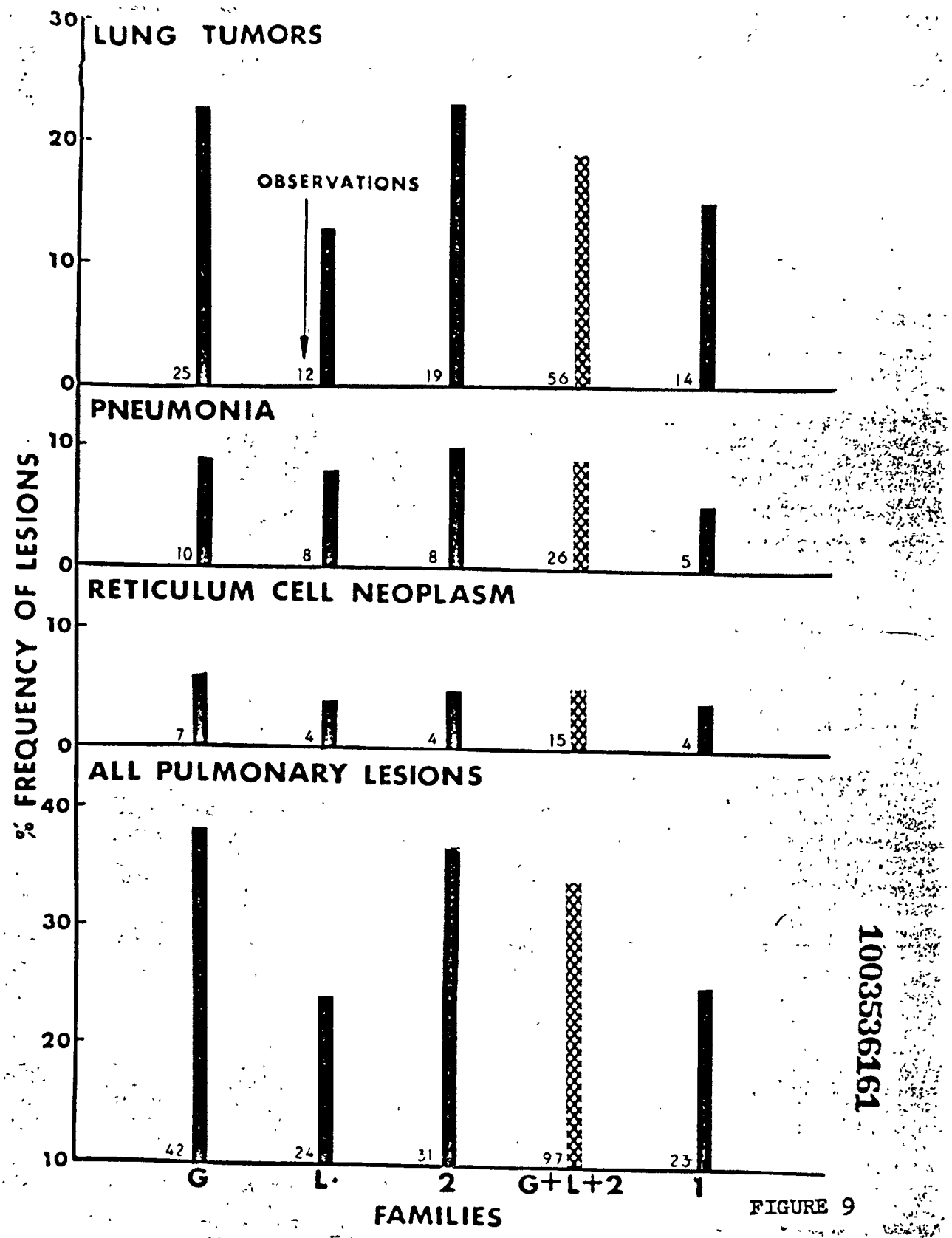


FIGURE 9

100353161

Text - Figure 10

Comparative frequency of occurrence of severe (3+ or 4+) emphysema with concurrent lung lesions, by age group, in High (Families G, L and 2) and Low Pulmonary Emphysema Incidence Families.

The number at each point is the

$$\left(\frac{\text{number with severe emphysema and pulmonary lesion(s)}}{\text{total observed}}\right)$$

for each age group. Each age group spans a time of 3 months.

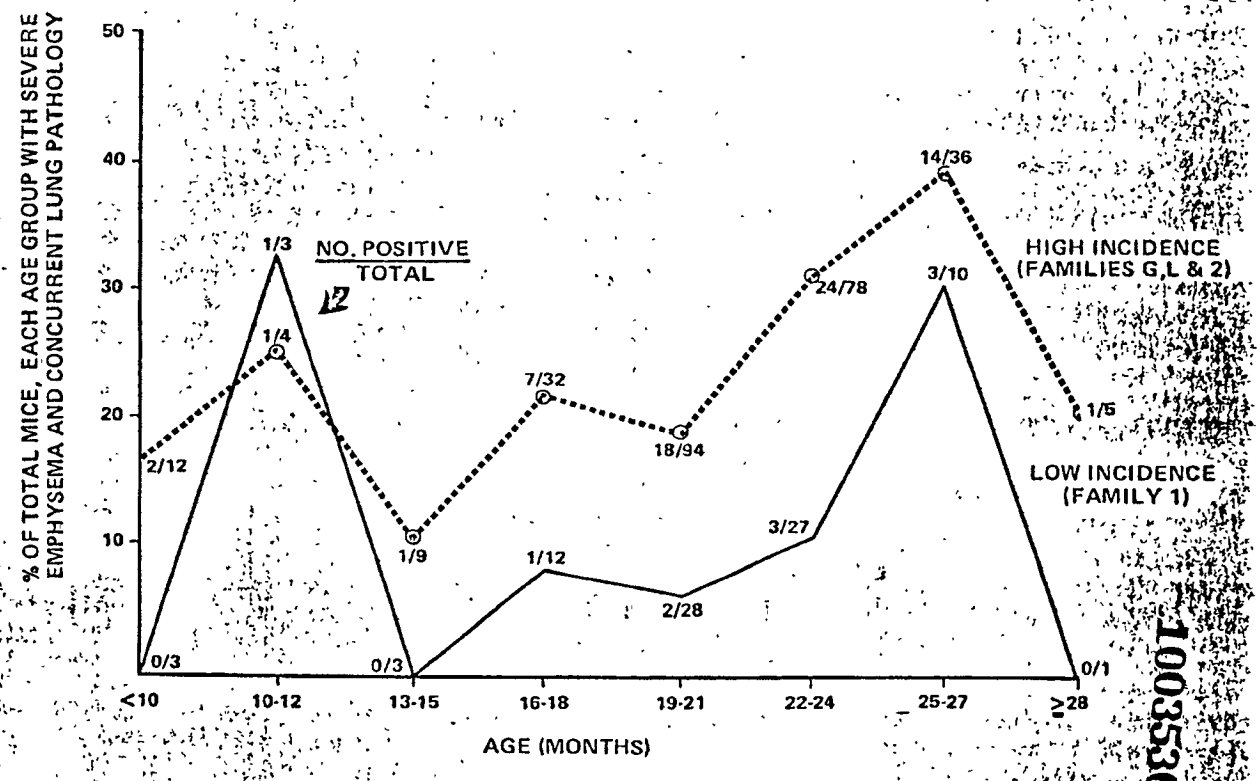


FIGURE 10

Text - Figure 11

Comparative alpha$_1$ trypsin inhibition between male and female mice of the different families.

Each bar indicates the mean of all male or female mice in each group. The mean of the High Incidence Families (G, L and 2) is shown in a separate bar.

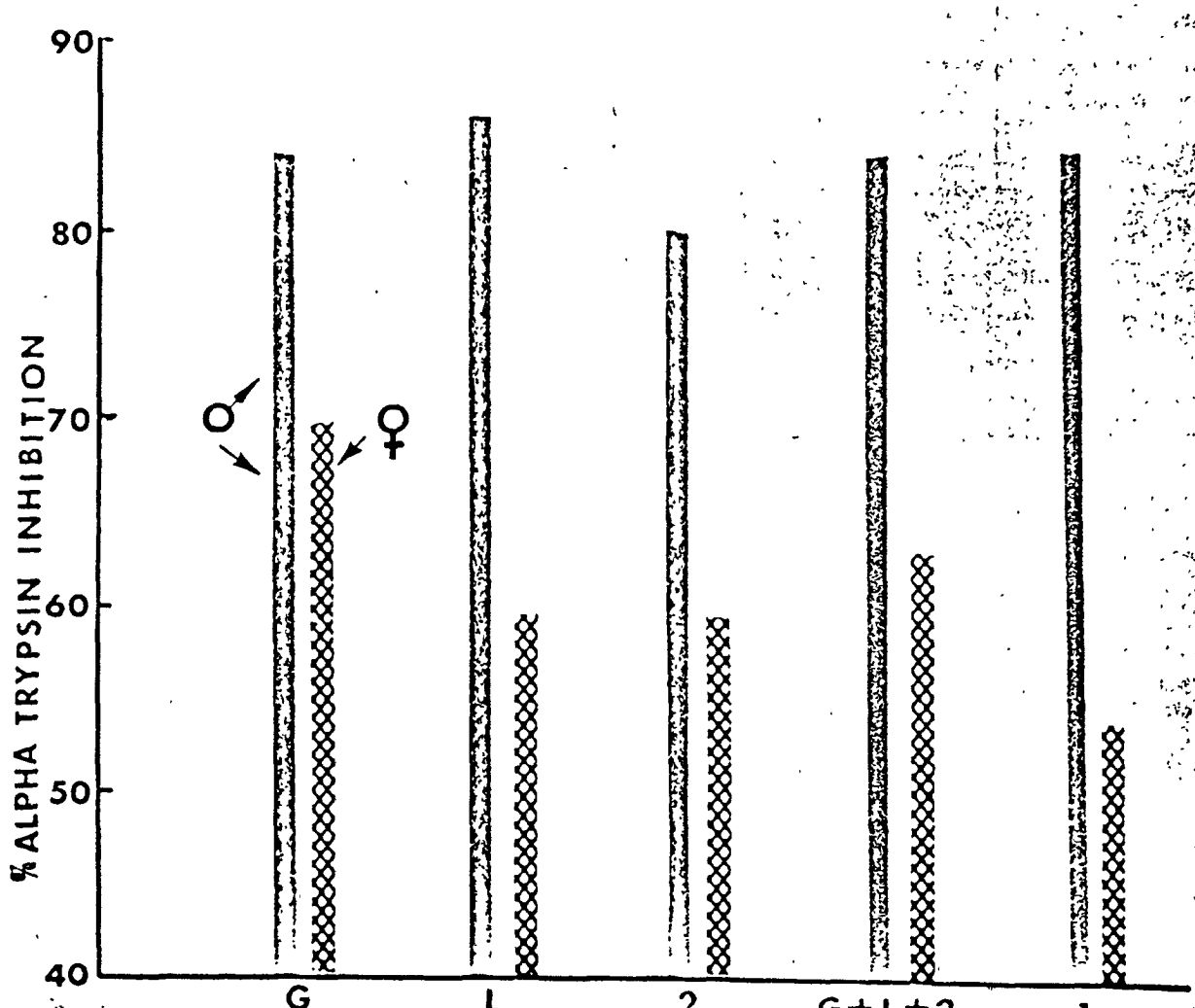


FIGURE 11

1003536165

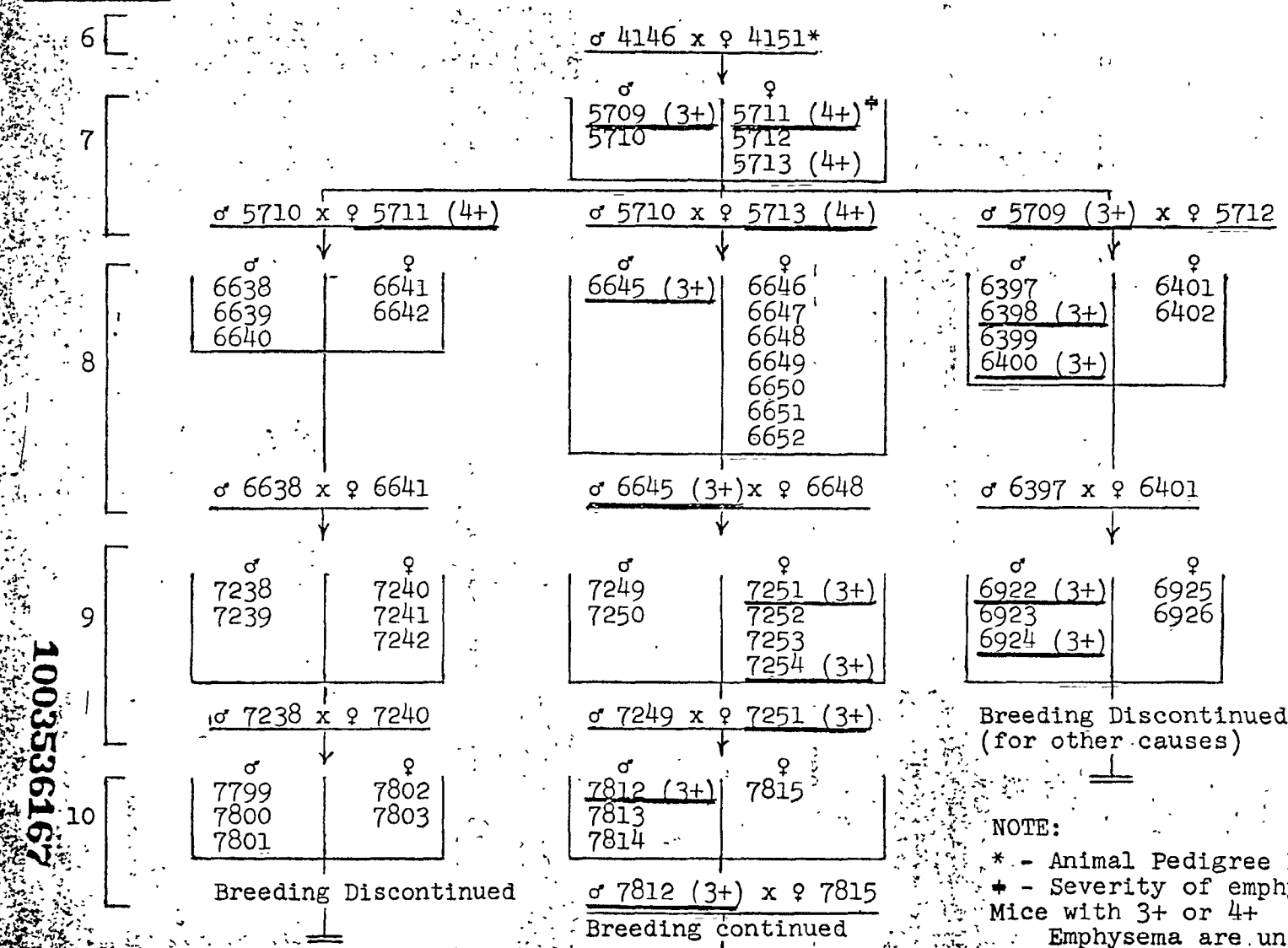
Text - Figure 12

Excerpt from the Pedigree chart of Family L, illustrating the segregation of a tendency toward severe emphysema within a family.

Underneath each mating, the individual mice comprising the progeny are shown by pedigree number and sex. Mice later diagnosed as having 3+ or 4+ emphysema are underlined. Note that in Generation 7, ♂ No. 5710 was bred to 2 of his sisters (♀ No. 5711 and ♀ No. 5713).

This figure illustrates the fact that since a diagnosis of severity of emphysema is established only after the mouse has died, usually well past breeding age, selection of breeder matings must be based on retrospective data of their ancestors, 3 to 5 generations past. The figure illustrates that even this information is of great value in attempting to accentuate the trend toward a high or low incidence of pulmonary emphysema. Physiological or biochemcial measurements which prove to be predictive of probable future emphysema during the active breeding age of the mice would be of immense value in such genetic studies.

FIGURE 12 - Excerpt from Pedigree chart of Family L



NOTE:
* - Animal Pedigree No.
+ - Severity of emphysema
Mice with 3+ or 4+ Emphysema are underlined.